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Evaluation of anti-inflammatory effect of derivative (*E*)-*N*-(4-bromophenyl)-2-(thiophen-2-ylmethylene)-thiosemicarbazone

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ABSTRACT

The present study aimed to further investigate the anti-inflammatory activity of (*E*)-*N*-(4-bromophenyl)-2-(thiophen-2-ylmethylene)-thiosemicarbazone (BTTSC) as well as its antinociceptive effects. The anti-inflammatory activity was assessed using the model of ear edema induced by croton oil-induced and also evaluated in models of paw edema carrageenan-induced and by compound 48/80. Evaluation of the antinociceptive effect was performed through formalin test. In the nociception test induced by formalin the BTTSC showed activity in both phases of the pain, highlighting inflammatory pain, where it was able to reduce the time to paw lick 62.3, 84.30 and 100% at doses of 30, 100 and 300 mg kg^{-1}. The anti-inflammatory activity was performed ear edema induced by croton oil, where none of the doses tested was capable of significantly regress edema. The paw edema carrageenan-induced showed activity compound, where the edema was reduced by 81.9 and 83.2% in the first two times of the experiment at the highest dose used. The paw edema assay induced by compound 48/80, showed that BTTSC after 15 min of the inoculum phlogistic agent showed significant reduction of edema with values of 56.53% at a dose of 30 mg kg^{-1}. Our results suggesting this compound exerts its antinociception effects connected with peripheral mechanisms. Furthermore, the compound was able to act in two phases of inflammation carrageenan-induced, highlighting the initial phase. This suggests an action on the early mediators of inflammation. The paw edema assay induced by compound 48/80 confirmed our hypothesis indicating action of the compound via histamine.

1. Introduction

Inflammation is a physiological response to injury and infectious agents, such as viruses and microbes [1]. The central role of the inflammatory response is played by mast cells that are responsible for conducting a series of intracellular signaling, activation of arachidonic acid (AA) and its subsequent metabolism of prostaglandins and leukotrienes, by way of cyclooxygenase (COX) and lipoxygenase (LOX), respectively. These responses contribute to the inflammatory response [2].

The cardinal signs of inflammation, redness (rubor), swelling (tumor), heat (calor) and hyperalgesia (dolor), develop as an acute response to a local inflammatory insult [3]. These symptoms result from the action of inflammatory agents such as bradykinin, serotonin, histamine, prostaglandins, leukotrienes and nitric oxide, which can originate locally or from cells that infiltrate in the site of insult [4,5].

Although several anti-inflammatory drugs are available, many of them can cause side effects after long-term use [6]. Thus, studies aiming at the discovery of new anti-inflammatory therapeutic agents has been the focus. In this context, the thiosemicarbazones appear as perspective since this group of molecules are associated with diverse biological activities such as antituberculosis [7], anti-*T.cruzi* [8], antibacterial and antifungal [9], antitumor [10] and anti-inflammatory [11,12].

We previously demonstrated antitumor activity thiosemicarbazone derivatives, where the compound 7 (BTTSC as reported herein) (Fig. 1) is highlighted and presented to the activity against

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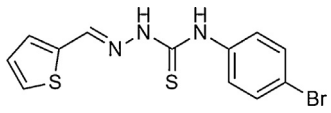

Fig. 1. Structure of derivative (*E*)-*N*-(4-bromophenyl)-2-(thiophen-2-ylmethylene)-thiosemicarbazone (BTTSC).

Ehrlich's paw model, a breast adenocarcinoma [13]. Ehrlich tumor cells generate an inflammatory response that leads to increase in vascular permeability, edema formation and cell migration [14]. In this study, treatment with 30 mg kg^{-1} of BTTSC inhibited tumor development inhibited from the 6th day to the 15th day of treatment, inhibiting in 71.1% (15th day), in comparison to negative control group [13]. Thus, it can be inferred that the anti-tumor action of BTTSC may have also occurred through modulation of inflammation mediators.

Therefore, the objective of this study was to investigate the effect of BTTSC in models of inflammation induced by different mediators. As inflammation and nociception are closely related, the antinociceptive activity of BTTSC was also evaluated, for thus collaborate with the various therapeutic inflammatory processes.

2. Results and discussion

The acute toxicity of the derivative was previously reported, where no toxic effect was shown. Thus, the doses used in the experiments were the same (30, 100 and 300 mg kg^{-1}) [13].

The formalin test is the most widely used for screening new compounds with antinociceptive activity because it is what most resembles clinical pain when compared to other tests. This test of nociception is biphasic that involve different mechanisms. The first phase of pain is attributed to direct activation of nociceptors and primary afferent C fibers by formalin, causing release of bradykinin and substance P [15,16]. The second phase of nociception is believed to be due to an inflammatory reaction that release prostaglandins, serotonin, histamine and bradykinin [17].

In this model, the thiophene-thiosemicarbazone derivative (BTTSC) inhibited the licking response of mice in both phases. In the first phase, the highest dose was effective in significantly decreasing the licking time 73.7% (saline: 103.6 ± 11.87, BTTSC 300 mg kg^{-1}: 27.25 ± 10.77). In the second phase, the BTTSC has demonstrated activity in all evaluated doses, 30; 100 and 300 mg kg^{-1}, being able to reduce the licking time 62.3; 84.3 and 100%, respectively, showing a dose-dependent effect of this compound on an inflammatory process algesia (saline: 173.5 ± 23.18, BTTSC 30 mg kg^{-1}: 64.25 ± 24.93, BTTSC 100 mg kg^{-1}: 27.25 ± 17), suggesting this compound exerts its antinociception effects connected with inhibition of inflammatory mediators (Fig. 2).

As the BTTSC was active in the inflammatory phase of the formalin test, we decided to investigate the anti-inflammatory potential of this new class of compounds. Thus, it was initially used the experimental model of ear edema induced by croton oil. The Fig. 3 shows the results obtained by the assay used.

None of the doses tested (30, 100 and 300 mg kg^{-1}) were able to significantly regress caused edema in ear of mice induced by such phlogistic agent. However, it can not be concluded that the 2-thiophene-thiosemicarbazone derivative has no anti-inflammatory activity, since the ear edema model induced by croton oil leads to activation of phospholipase A_2 [18] this compound can act in the inhibition of other mediators of inflammation.

The 12-*o*-tetracanoylphorbol esters, substance that causes irritation resulting from the application of croton oil causes intense vasodilation as a result of increased vascular permeability and blood flow induced by arachidonic acid metabolites [19,20]. At inflammation caused by croton oil, PLA_2 inhibitors (corticosteroids) and lipooxigenases (LOX) demonstrated better activity in reducing edema in the ear. Compounds having such mechanism of action the inhibition of the enzyme cyclooxygenase (COX) or antihistamines have little or no effect in this model of evaluation [21].

In order to deepen the studies of anti-inflammatory activity of the BTTSC, we decided to evaluate it against the experimental model of paw edema induced by carrageenan. It is possible to observe the graphic below that the BTTSC dropped significantly paw edema in the first 4 h of evaluation when compared to the negative control. The edema was reduced by 81.9 and 83.2% in the first two times of the experiment (1 and 2 h, respectively) at the highest dose used (control: 18.85 ± 2.63, 32.86 ± 4.97 and BTTSC 300 mg kg^{-1}: 3.41 ± 1.4; 5.53 ± 1.96) (Fig. 4).

In addition, after 4 h of inoculum carrageenan the derivative was able to contain the inflammatory peak shown by the negative control, regressing edema in rat paw at 60.33% (control: 49.99 ± 5.84; BTTSC 300 mg kg^{-1}: 19.83 ± 4.4). The positive control demonstrated at the same time, regression of edema similar to derivative presenting 50.15% decrease of the inflammatory process (24.92 ± 4.11). The remaining doses used in this experiment not produced significant reductions in paw edema in mice (Fig. 4).

The carrageenan-induced inflammation is a biphasic process. In the initial phase, up to 2 h after injection of the phlogistic agent, it is determined by an initial vasodilation mediated by the release of mediators such as histamine and serotonin. The later stage, up to 6 h after the injection of carrageenan, is mediated primarily by kinins, nitric oxide, prostaglandins, derived from the neutrophil recruitment [4,5]. The results of this experiment revealed a BTTSC activity in both phases of inflammation induced by carrageenan, or a derivative acting in the early mediators of inflammation or through inhibition of pro-inflammatory cytokines.

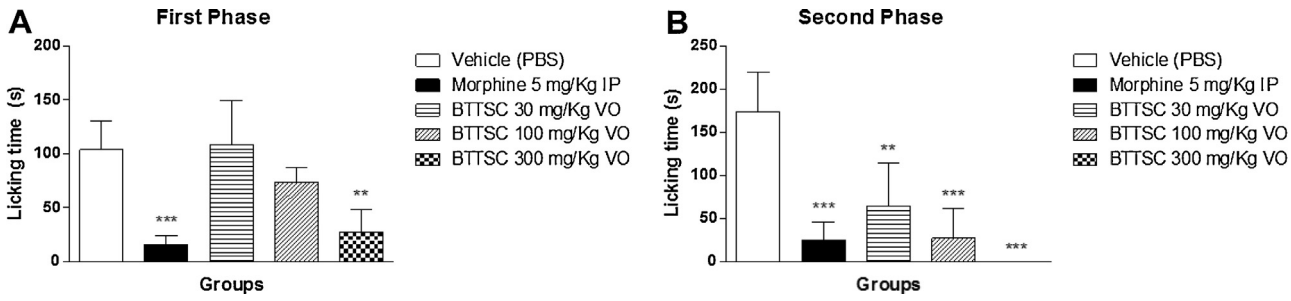

Fig. 2. Effects of BTTSC on test algesia induced by formalina, indicating the first and second phases of nociception. Data were expressed as the mean ± SE of 5 animals per group. Results were considered significant when $^{**}p < 0.01$ and $^{***}p < 0.001$, determined by analysis of variance (ANOVA) followed by Tukey test.